

## **Drosha but not Dicer regulates hMSCs cell cycle progression through a miRNA independent mechanism**

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Human multipotent stromal cells from bone marrow (hMSCs) are progenitor cells capable of differentiating into a variety of mature tissues including osteoblasts and adipocytes as well as other cellular phenotypes. The cells hold great promise for therapy but the molecular mechanisms that govern the survival, proliferation and differentiation remain unclear. The existence and function of a class of small non-coding RNA molecules known as microRNAs (miRNAs) has gained recent attention as regulators of gene expression during germ line development and cellular differentiation. Recently we demonstrated that the miRNA regulates hMSC differentiation. To determine the role of the miRNA pathway in hMSC proliferation, Drosha and Dicer knockdown hMSCs were generated using a lentiviral based tetracycline inducible sh-RNA. hMSCs with reduced Drosha expression had a significantly reduced proliferation rate, while hMSCs with reduced Dicer expression displayed a proliferation rate similar to untransduced cells. Cell cycle analysis identified that unlike Dicer knockdown, Drosha knockdown hMSCs contained an increased number of G1 phase cells, with a reduced level of cells in S phase, compared to controls. ELISAs of hMSCs revealed decreased levels of pRB and stable levels of total RB with Drosha knockdown. Two key regulators of the G1/S phase transition, cyclin dependent kinase inhibitor 2A (p16) and cyclin dependent kinase inhibitor 2B (p15), were increased in Drosha knockdown cells but not in Dicer knockdown. Transcripts of 28S and 18S rRNA were significantly reduced in Drosha knockdown hMSCs, with no change in rRNA levels in Dicer knockdown hMSCs. 45S pre-rRNA transcripts were not significantly different in either knockdown model. The above results indicate that Drosha modifies hMSC proliferation through a miRNA independent mechanism, potentially by regulating rRNA processing.

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